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REDUCED VIGOUR, CHLOROPHYLL DEFICIENCY, AND OTHER EFFECTS OF SELF-FERTILIZATION IN *PINUS*[1]

BY L. P. V. JOHNSON[2]

Abstract

Seedlings derived from self-pollinations in *Pinus Strobus* and *P. sylvestris* were, at four years of age, significantly smaller than comparable seedlings derived from open and intraspecific cross-pollinations. In *P. Strobus*, 11 out of 46 seedlings of selfed origin showed pronounced chlorophyll deficiency. Selfing had an unfavourable effect on seed set and seedling emergence in *P. sylvestris* and on seed set in *P. resinosa*.

The results are discussed in relation to the occurrence of chlorophyll deficiency in pine and in relation to the silvicultural practice of seed-tree cutting. It is recommended that, in estimating the numbers of seed trees required to re-seed a cut-over area, silviculturists take into consideration the provision of adequate opportunity for cross-pollination between individuals of the same species.

Introduction

In the normal course of investigations on interspecific hybridization of forest trees, it has been a practice in some genera to extend the work to include controlled self-pollination and intraspecific cross-pollination. The results reported herein were obtained from seedlings derived from such work performed in 1939 at the Petawawa Forest Experiment Station on white pine (*Pinus Strobus* L.), Scotch pine (*P. sylvestris* L.), and red pine (*P. resinosa* Ait.).

Methods

To effect controlled pollinations, unopened female flowers were covered with double glassine bags and, upon reaching the receptive stage, were pollinated with appropriate pollen by removing the outer bag, puncturing the inner bag with a pointed glass tube, blowing the pollen over the flowers, sealing the puncture, and replacing the outer bag.

Intraspecific crossing was included in the procedure in order to provide materials derived from cross-fertilization that would be in every way comparable to those from self-fertilization with respect to effects of bagging, etc.

In the fall of 1940 the cones developed from these operations, together with cones from natural (open) pollination of the same trees, were collected, the seed extracted, stratified, and sown in a nursery bed in the spring of 1941.

[1] *Manuscript received June 5, 1945.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Part of a co-operative project of the Subcommittee on Forest Tree Breeding, Associate Committee on Forestry. N.R.C. No. 1320.

[2] *Geneticist.*

Seedling emergence counts were made during the summer of 1941. After two years in the bed the seedlings were transplanted to the nursery in May, 1943, with spacing of 12 in. in rows 3 ft. apart. Spread and height measurements were made in August, 1944, and weights were taken in April, 1945, at the time of transplanting to a permanent site at the Provincial Forest Station, Orono, Ont.

Results

Seed set and seedling emergence data for the various types of fertilization are compared in Table I. White pine showed no appreciable difference in seed set for open-, cross-, or self-pollinated cones. Scotch and red pines, however, showed a marked reduction in seed set for self-pollinated cones. Emergence data tended to follow the seed set results: percentage emergence of white pine seedlings was about the same for all types of fertilization, while that of Scotch pine seedlings dropped very low for seeds from self-pollinated cones.

TABLE I

SEED SET AND SEEDLING EMERGENCE FROM CONTROLLED SELFING, CONTROLLED CROSSING, AND OPEN POLLINATION OF CONELETS OF WHITE PINE (*W*), SCOTCH PINE (*S*), AND RED PINE (*R*). CONES COLLECTED 1940

Material	No. of bags involved	No. of cones collected	No. of seeds	Seed set[1], %	No. of seedlings emerged	Emergence, %
W-1, open pollinated	—	5	191	95	67	35
W-1 × *W*-2	2	3	122	97	38	31
W-1, selfed	3	5	190	95	54	28
S-1, open pollinated	—	5	76	60	36	47
S-1 × *S*-2	3	1	18	72	8	44
S-1, selfed	4	5	40	31	4	10
R-1, open pollinated	—	23	625	99	—[2]	—
R-1 × *R*-2	9	14	304	84	—	—
R-1, selfed	12	8	119	45	—	—

[1] *Percentage of seed set calculated from counts on number of seeds and functional seed scales.*

[2] *Emergence data not given since pre-emergence damping-off of seedlings made accurate observations impossible.*

Owing to the high mortality from damping-off among red pine seedlings, and to the possibility that this mortality might be of a selective nature, red pine materials were not included in the later phases of the study.

Average measurements of four-year-old seedlings derived from the various types of fertilization, together with standard deviations, are given in Table II. The results demonstrate, in both white and Scotch pines, that seedlings of selfed origin were markedly smaller in mean spread and height and lower in mean weight as compared with seedlings from open and cross-pollinations.

As the seedlings developed in the bed it was noticed that about one-quarter of the selfed progeny of white pine (*W*-1) showed marked chlorophyll deficiency. After being transplanted to the nursery these subnormal individuals

PLATE I

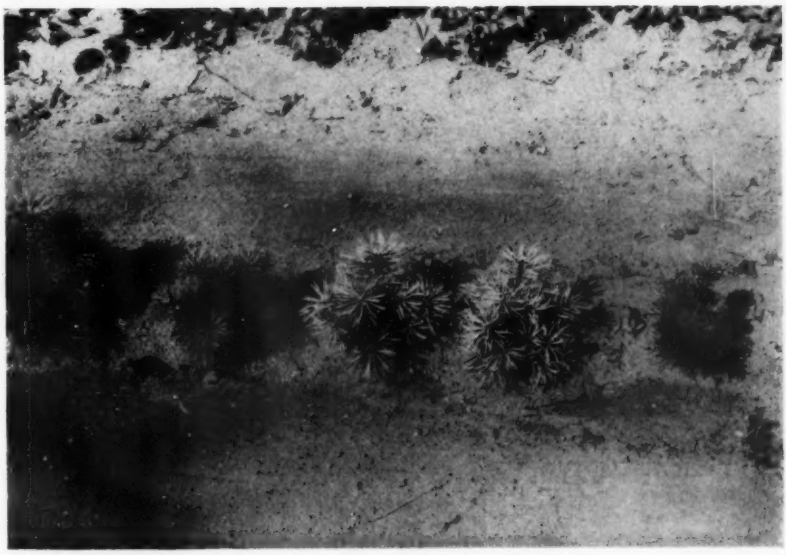

FIG. 1. *Chlorophyll-deficient and full-green seedlings in white pine material of selfed origin.*

TABLE II

MEAN VALUES FOR CERTAIN QUANTITATIVE CHARACTERS OF FOUR-YEAR-OLD WHITE PINE (*W*) AND SCOTCH PINE (*S*) SEEDLINGS DERIVED FROM THE VARIOUS TYPES OF FERTILIZATION, TOGETHER WITH STANDARD DEVIATIONS

Material	No. of seedlings	Means			Standard deviations		
		Spread[1], in.	Height, in.	Weight, gm.	Spread, in.	Height, in.	Weight, gm.
W-1, open pollinated	27	7.7	8.5	121	1.2	1.7	32
W-1 × *W*-2	27	7.0	7.9	114	1.4	2.3	41
W-1, selfed, total	46	5.0	6.2	64	1.7	1.9	—[2]
W-1, selfed, full-green	35	5.0	6.6	75	1.7	1.8	34
W-1, selfed, chlorophyll-deficient	11	4.0	4.7	30	1.7	1.6	16
S-1, open pollinated	10	19.5	22.1	624	3.9	4.9	261
S-1 × *S*-2	8	23.4	23.6	609	1.3	2.3	200
S-1, selfed	4	14.9	14.8	323	3.4	3.0	195

[1] *Spread represents the combined length of two typical and opposite branches of the lowest whorl.*

[2] *The variability among selfed, full-green seedlings was so much greater than that among selfed, chlorophyll-deficient seedlings that calculation of a standard deviation for the combined material was not considered valid.*

were under regular observation during two seasons. The new growth of needles in the early spring was white to cream colour and stood out in contrast to the almost normal green of the previous season's growth. As the season advanced the colour of the new growth changed through shades of yellowish-white and yellowish-green to a green of low intensity in the fall.

Data in Table II demonstrate that, in both white and Scotch pines, seedlings of selfed origin showed marked reductions, on the average, in spread, height, and weight as compared to seedlings from open and cross-pollinations.

In Table III the various materials are compared statistically and the differences in spread, height, and weight expressed as t values.

No significant differences between materials of open- and cross-pollinated origin were apparent, except the greater spread in material from cross-pollinated Scotch pine. This shows that the artificial procedure involved in effecting self- and cross-pollinations did not significantly contribute to reduction in vigour of growth.

In white pine all comparisons between materials of open- and cross-pollinated origin on the one hand, and of selfed origin on the other, show highly significant differences. This demonstrates strikingly that selfing in white pine produced marked reduction in vigour. The t values for weight prove that this was true irrespective of whether the chlorophyll-deficient seedlings were included in the selfed material.

In white pine seedlings of selfed origin, vigour of growth in the chlorophyll-deficient type was significantly less than in the full-green type.

Scotch pine seedlings of open- and cross-pollinated origin tended to show significantly greater dimensions and weight as compared to seedlings of selfed

TABLE III

THE *t* VALUES FOR COMPARISONS BETWEEN QUANTITATIVE CHARACTERS OF WHITE PINE (*W*) AND OF SCOTCH PINE (*S*) SEEDLINGS

Comparison	*t* values		
	Spread	Height	Weight
W-1, open pollinated vs. *W*-1 × *W*-2	1.77	1.30	0.74
W-1, open pollinated vs. *W*-1, selfed, total[1]	7.60**	5.16**	5.39**
W-1 × *W*-2 vs. *W*-1, selfed, total[1]	5.61**	3.70**	4.07**
W-1, selfed, full-green vs. *W*-1, selfed, chlorophyll-deficient	2.67**	2.94**	—[2]
S-1, open pollinated vs. *S*-1 × *S*-2	2.72*	0.81	0.13
S-1, open pollinated vs. *S*-1, selfed	2.05	2.76*	2.06
S-1 × *S*-2 vs. *S*-1, selfed	6.50**	5.76**	2.35*

* *Denotes 5% level of significance.*

** *Denotes 1% level of significance.*

[1] *"Selfed, total" applies only to t values for spread and height; t values for weight are based on "selfed, full-green."*

[2] *Since it was not valid to calculate a standard deviation for the combined, selfed data, no t value could be obtained.*

origin. The fact that statistical significance is not so marked as in white pine may be attributed to the smaller numbers involved (see Table II).

Discussion

The occurrence of chlorophyll-deficient individuals in pine is apparently quite rare. A recent report from Sweden by Degelius (1) describes a young Scotch pine, half a metre in height, with a yellowish-white new growth that changed to a greenish colour later in the season. Degelius assumed that this subnormal form was due to genetical rather than environmental factors. The present work shows that the origin of these forms is, at least in some instances, related to self-fertilization.

Chlorophyll-deficient plants are of fairly common occurrence in nature, especially when normally cross-fertilized species are selfed. The genetic interpretation is that such selfing produces homozygosity of defective genes thus permitting the expression of characters such as albinism, low vigour, etc. In the present results the proportion of normal to chlorophyll-deficient seedlings (35 : 11) is very close to the monohybrid segregation ratio (3 : 1).

No literature has been found on the effects of self-fertilization in pine relative to development of the resulting progeny. However, the work of Sylvén and Langlet on spruce, reported by Langlet (2) is relevant. Their results show that seeds produced by selfing are of lower germinability than those from open pollination and that seedlings from selfing have a greatly reduced capacity to survive and a growth rate less than half that of seedlings from open pollination. These results agree essentially on all points with those for

Scotch pine in the present work and, on the main point, growth vigour, are in agreement with the results for white pine.

It is considered that the present results have a practical bearing on the silvicultural logging practice known as seed-tree cutting, in which a few widely spaced, individual trees are left standing to re-seed the cut-over area. Westveld (3), presumably on the basis of range of seed dispersion, recommends as few as one loblolly or shortleaf pine and two western white pines per acre as seed trees. In dealing with ponderosa pine the same author recommends four seed trees per acre if there is no established seedling reproduction and correspondingly fewer as varying amounts of reproduction exist. It is believed that such spacing would reduce cross pollination between individual seed trees to a point where the resulting, normally vigorous seedlings would not be able completely to eliminate the weaker but more numerous seedlings of selfed origin.

It is concluded that, in estimating the numbers of seed trees necessary to re-seed an area, silviculturists must among other considerations take into account the provision of adequate opportunity for cross pollination between individuals of the same species.

Acknowledgments

The author is indebted to Mr. D. B. W. Reid for the statistical work and to Dr. J. M. Armstrong and Mr. M. W. Thistle for reviewing the manuscript.

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VEGETATIVE PROPAGATION OF CONIFERS

XIII. ROOTING OF NORWAY SPRUCE CUTTINGS IN THE GREENHOUSE[1]

By N. H. Grace[2] and J. L. Farrar[3]

Abstract

Greenhouse propagation of Norway spruce cuttings in various media containing sand and peats resulted in 80% rooting in a peat-humus–sand medium. Canadian peat moss and Swedish peat media were inferior but the weekly addition of potassium acid phosphate and magnesium sulphate solutions to a Swedish peat medium resulted in the rooting of 96% of the cuttings. Beneficial effects of peat humus were related to available nutrient materials. Peat humus extracted with methyl alcohol and acetic acid did not affect rooting, but sodium hydroxide extraction, hot water extraction, and air drying at 110° C. were harmful. Propagation under suboptimum conditions disclosed markedly beneficial effects from mixtures and mulches of peats, soil, and active silica, the active silica being particularly favourable in the mulched condition.

Medium cuttings, 3 to 6 in. long, rooted 96%, and long cuttings, 6 to 10 in., 71%. Uncovered cuttings and those covered with celloglass were superior in their responses to those under either cheesecloth or factory cotton screens.

Plant hormone chemicals, when applied in a talc carrier dust, tended to reduce rooting of Norway spruce cuttings; the injurious effects increased progressively with concentration over the range from 500 to 4000 p.p.m. Indolylbutyric acid showed neither damaging nor beneficial effects. Naphthylbutyric acid and potassium naphthylhexoate, while similar in effect, tended to be less injurious than naphthylacetic acid when used over a range of equivalent molecular concentrations. Application of naphthylbutyric acid at the 2000 p.p.m. level in a charcoal carrier resulted in rooting of 98% of the cuttings. Mean root length was increased by the application of 50 p.p.m. each of thiamin and nicotinic acid. While nutrient salts and naphthylbutyric acid, each separately, tended to reduce mean root length, the combination had no injurious effect.

Introduction

Heretofore spruce and pine cuttings in the greenhouse have yielded indifferent results with respect to rooting (8, 9, 11, 16, 17). However, high rooting percentages have been obtained from outdoor propagation of cuttings of certain conifer species (4-6, 20). Factors such as propagation medium, developmental stage at which the cutting is taken, position of the parent branch on the tree, part of the branch from which the cutting is made, and chemical treatments have been shown to affect rooting in outdoor frames, with media appearing to have greatest effect (3, 4, 6, 20). All of these factors have been shown to be important under greenhouse conditions (2, 8, 9, 11, 12, 16, 17, 21). Consequently study of media effects is an obvious approach to greenhouse propagation.

[1] *Manuscript received in original form, January 18, 1945, and as revised, June 12, 1945.*

Contribution from the Division of Applied Biology, National Research Laboratories, and the Dominion Forest Service, Ottawa. Part of a co-operative project of the Subcommittee on Forest Tree Breeding, Associate Committee on Forestry. Issued as N.R.C. No. 1319.

Part of the material contained in this communication was presented to Section V, Royal Society of Canada, May, 1945.

[2] *Biochemist, National Research Laboratories, Ottawa.*

[3] *Flight Lieutenant, Royal Canadian Air Force, Overseas; formerly Forest Engineer, Dominion Forest Service, Ottawa, Canada.*

While treatment of Norway spruce cuttings with phytohormone chemicals has tended to be injurious to rooting, some beneficial effects have been noted (12, 20). This communication describes the results of experiments in which Norway spruce cuttings were propagated in the greenhouse in a number of media, subjected to differing conditions, and treated with phytohormone chemicals, nutrient salts, thiamin, and nicotinic acid.

Experimental

Norway spruce (*Picea Abies* (L.) Karst.) cuttings were prepared from branches taken from the lower third portion of trees at the Petawawa Forest Experiment Station, Chalk River, Ont., by methods that have been previously described (17). Freshly prepared cuttings, of full length of the current year's growth and without a heel of old wood, were planted to a depth of from 1 to 2 in., depending on the size of the cutting. The media, two volumes of builder's sand and one volume of peat humus or other material under test (4, 6, 20, 22, 24), were placed to a depth of at least 6 in. in propagation frames in the greenhouse, where the temperature ranged around 65° F. during the winter months and until April and to a maximum of about 85° F. thereafter. Propagation frames were covered with factory cotton shades and the cuttings were lightly sprinkled with tap water daily. The medium was thoroughly drenched with tap water once a week. On sunny days, humidity in the frames was maintained by sprinkling the shades also with water.

All experiments were replicated and the treatments assigned within the replicates in a random manner. Results were subjected to analyses of variance except where the data were too meagre to warrant such treatment. The measurements taken are indicated in the tables and have been previously described (3-6, 8, 9, 11, 12, 16, 17, 19-21). All data on numbers of cuttings were subjected to angular transformation prior to statistical treatment (1). The experiments involved planting of 4540 cuttings.

Part I. Effects of media, length of cutting, and shading on the greenhouse propagation of Norway spruce cuttings

Materials and Methods

Class of Cutting and Shading Conditions

Branches were collected Dec. 10, 1940, packed tightly in cardboard cartons, and held outside under winter conditions. After the frozen branches were thawed, cuttings were prepared and separated into three length classes, 1.5 to 3, 3 to 6, and 6 to 10 in., and were planted immediately in compartments with four types of covering: open, celloglass, cheesecloth, and factory cotton. Compartments were 48 in. long by 10 in. wide and were separated by means of celloglass partitions 12 in. high placed flush to the surface of the media, segregating compartments as to humidity conditions but maintaining relative uniformity in the lighting. Coverings fitted tightly over the compartments. The three classes of cuttings were randomized as to position within each

compartment and compartments were randomized within each of the four replicate blocks. There were 10 cuttings to the group. Cuttings were planted Dec. 18, 1940, and removed for examination May 12, 1941; all rooted cuttings were then planted in the field.

Composition of Media

The experimental arrangement involved two propagation frames each containing 60 glazed earthenware crocks provided with an opening for drainage (9, 17). Cuttings, from the collection of branches previously described, varying in length from 3 to 6 in. were made and planted on Dec. 17, 1940. The media are described in Table III. It had already been determined that peat humus was very markedly superior to Swedish peat for rooting of Norway spruce and other conifers (3, 4, 6). Consequently, the selection of media and treatments thereof were such as to indicate possible differences in the imported peat, or by heat and extractive treatments to inactivate or remove the beneficial factors of the domestic peat humus. One of the extracts from peat humus was added to a Swedish-peat–sand medium. All nutrient treatments in this and other experiments made use of all, or part, of the mixture of nutrients known as Hoagland's (9, 23). The experiment involved 20 groups, but only 19 different media as the control Swedish-peat–sand mixture occurred twice in the series. There were 15 cuttings to the group and six complete replicates of the 20 treatment groups. Cuttings were removed from the media for examination on May 15, 1941.

Prior to planting cuttings, the number of bacteria and fungi in a few of the media were estimated* on albuminate agar incubated at room temperature (Table IV). While nitrifying bacteria were present in unsterilized media they were not apparent after sterilization. At the close of the experiment, the number of organisms was determined for a few media.

Two weeks after the cuttings were planted, samples of media to a depth of 2 in. were taken, moistened with boiled distilled water, and the pH determined. Similarly, after the cuttings had been removed, final pH values of the media were determined.

Mulched and Mixed Media

Branches were collected Nov. 24, 1941, and stored outside under burlap until Jan. 5, 1942, when cuttings were made and planted. Cuttings were 3 to 6 in. long and the experimental design included further use of a portion of the arrangement already described. There were four replications of the 15 media treatments with 15 cuttings to each. Cuttings were removed and measurements taken June 1, 1942.

The media are described in Table VI. The peat humus and Swedish peat have already been described. Petawawa** peat humus came from the

* *Counts were made by Dr. N. E. Gibbons, Bacteriologist, Division of Applied Biology, National Research Laboratories, Ottawa.*

** *Dr. Carl Heimburger, Dominion Forest Service, Ottawa, located the Petawawa peat bog and supplied the analytical data.*

Petawawa Forest Experiment Station, Chalk River, Ont. It was of interest that a comparison be made of this peat humus with others shown to be suitable for the vegetative propagation of conifers. This is a very well decomposed material with a pH range of 5.4 to 5.7 and has a relatively high mineral content, judging by its loss on ignition, which gave values ranging around 50%. Samples showed incomplete nitrification while the peat humus previously used nitrified completely (22, 24).

Media treatments were 15 in number: one, of sand alone; seven, in which peat, rich potting soil, or active silica* overlaid or mulched the sand to a depth of ½ in. after the cuttings had been planted; and seven, admixtures with sand. All cuttings were planted to a total depth of 1½ in. Propagation was conducted under suboptimum conditions, involving a weekly drenching of the medium and sprinkling of the cuttings every second day, sprinkling of the shades being omitted. The shades were removed six weeks after planting.

Results

Effects of Class of Cutting and Shading Conditions

Data for the average effects of size of cutting and covering of propagation frame are given in Table I. Medium sized cuttings rooted 96%. The 71% level of rooting reached by the large cuttings and the resulting number of roots and new growth shoots are of particular interest. The number of roots varied directly with length of cutting. On the average, cuttings without covering produced substantially more roots per rooted cutting than those under cover. The open and celloglass coverings were superior to both cheese-

TABLE I

AVERAGE ROOTING RESPONSES OF DIFFERENT CLASSES OF NORWAY SPRUCE CUTTINGS TO PROPAGATION UNDER VARIOUS SHADES

Response	Class of cutting				Covering of propagation frame				
	Small	Medium	Large	Necessary difference, 5% level	Cheese cloth	Factory cotton	Cello-glass	Open	Necessary difference, 5% level
Number of rooted cuttings									
Transformed data	76.1	83.9	58.8	9.1	—	—	—	—	—
%	90	96	71	—	—	—	—	—	—
Number of roots per rooted cutting	2.8	4.1	6.4	0.7	4.4	3.9	3.9	5.5	0.8
Mean root length, mm.	59	54	41	8.4	46	42	56	61	9.7
Number of new growth shoots per rooted cutting	1.1	1.2	2.7	0.33	—	—	—	—	—

** The active silica was kindly prepared by Mr. D. Wolochow, Division of Chemistry, National Research Council, Ottawa.*

cloth and factory cotton in respect of their effects on mean root length. Average and interaction effects of type of cutting and covering on mean length of new growth are shown by the data of Table II. Medium and large cuttings produced substantially longer new-growth shoots than did small cuttings. The celloglass covering was the most favourable while the other three coverings failed to differ significantly in their effects. The significance of the interaction related almost entirely to the favourable response of the large cuttings to the celloglass covering.

TABLE II

INTERACTION EFFECTS OF CLASS OF CUTTING AND SHADING ON MEAN LENGTH OF NEW GROWTH, MM.

Class of cutting	Kind of covering				Means for class of cutting
	Cheesecloth	Factory cotton	Celloglass	Open	
Small	19	21	22	21	21
Medium	28	28	27	24	27
Large	23	25	30	27	26
Means for coverings	23	24	26	24	—

NOTE:—*Necessary differences, 5% level, for interaction, 3.4; means of type, 1.71, and means of coverings, 1.95.*

Effects of Composition of Media

Analyses of variance indicated highly significant media effects in relation to number of rooted cuttings, number of rooted cuttings with new growth, number of roots per cutting, and mean root length. Data for the responses and also initial values for the pH of the media are given in Table III. Beneficial effects of peat humus were significantly reduced by air-drying at 110° C. for 15 hr., and by sodium hydroxide and hot water extraction. Rooting was generally improved in Swedish peat following the addition of nutrients, although calcium carbonate tended to have a deleterious effect. The weekly addition of potassium acid phosphate and magnesium sulphate resulted in 96% rooting, a value significantly above those for all other treatments. All peat media were markedly superior to sand alone, which yielded only 18% rooted cuttings. Grinding of the Swedish peat in a Wylie mill increased rooting, from about 54 to 73%. Treatment of Swedish peat (of sphagnum origin) with thiamin did not effect improvement. It is apparent that Canadian peat moss (sphagnum peat) was substantially similar to Swedish peat in its effects. Good rooting response followed when sand was mulched with ½ in. peat humus; the results were practically the same as from a mixture of the two components.

The effect of nutrient treatment on the formation of both new growth and roots calls for consideration. While the numbers of such cuttings were

TABLE III

RESPONSES OF NORWAY SPRUCE CUTTINGS TO GREENHOUSE PROPAGATION IN VARIOUS MEDIA

Media and treatment	No. of rooted cuttings		No. of roots per rooted cutting	Mean root length, mm.	No. of rooted cuttings with new growth		New growth shoots on rooted cuttings		pH of media two weeks after cuttings planted
	Transformed data	%			Transformed data	%	No. per cutting	Mean shoot lengths, mm.	
Swedish-peat–sand									
Watered weekly with 200 cc. 2 p.p.m. thiamin solution	42.3	46	2.9	98	18.4	12	1.0	15	7.60
Watered weekly with 200 cc. nutrient solution*	60.3	74	3.1	153	47.0	53	1.1	19	7.40
Watered weekly with 200 cc. KH_2PO_4 and $MgSO_4$*	83.1	96	3.5	168	57.6	71	1.2	20	7.37
Mixed with 4% of nutrient*	62.0	77	3.3	124	34.4	33	1.3	16	7.25
Mixed with 10% of peat humus by volume	55.5	67	3.4	124	32.0	29	1.0	15	7.63
Mixed with 2% of $CaCO_3$	39.7	41	3.2	133	23.2	19	1.3	13	7.60
Treated with methyl alcohol extract from 8 litres of peat humus	42.4	46	3.3	141	21.4	14	1.2	16	7.35
Peat ground in Wylie mill before mixing	59.3	73	4.1	148	34.3	32	1.2	17	7.75
No additional treatment	49.7	58	3.2	119	21.6	17	1.0	21	7.77
Peat-humus–sand, the peat having been:									
Sterilized at 120° C. for 2½ hr.	60.2	74	3.5	143	34.1	33	1.3	15	7.48
Air-dried at 110° C. for 15 hr.	36.5	37	3.0	101	17.0	13	1.1	16	7.87
Extracted with 5% acetic acid	60.2	74	3.6	134	41.0	43	1.1	16	7.37
Extracted with 0.5% NaOH	45.6	51	3.7	139	17.0	11	1.0	15	8.89
Extracted with 95% methyl alcohol	65.6	80	3.6	143	32.0	30	1.2	17	7.63
Extracted with water at 100° C.	54.6	66	3.5	112	30.4	30	1.2	15	7.80
Untreated	66.6	81	3.3	128	36.2	36	1.3	15	7.60
Sand only	23.8	18	2.0	76	5.0	2	1.0	18	8.77
Sand mulched with ½ in. peat humus	65.4	82	3.2	88	39.0	40	1.5	15	8.75
Sphagnum-peat**–sand	54.3	64	3.2	144	23.7	20	1.3	15	7.78
Swedish-peat–sand	44.0	49	3.1	117	18.4	16	1.1	18	7.55
Necessary difference, 5% level of significance	10.96	—	0.86	43	12.1	—	—	—	—

* *Nutrient salts as in Hoagland's solution.*

** *This is a peat of Canadian origin included for comparison with the Swedish peat.*

substantially increased there was no increase either in the number of shoots per cutting nor in the mean shoot length. Further, use of the balanced nutrient mixture tended to give poorer results than application of only potassium acid phosphate and magnesium sulphate.

Statistical treatment of the data on pH of the media disclosed significant differences among the initial values (Table III) and a significant relation between initial and final values. There was a tendency for the final values to be somewhat higher, probably owing to watering with tap water having a pH around 8. Determination of correlation coefficients between the first four responses in Table III and the initial pH values indicated that mean root length tended to decrease as the pH was raised ($r = .54^{**}$). A similar though less marked effect was shown by the number of rooted cuttings with new growth.

Bacteria and fungi counts are given in Table IV. After 14 days' culture the presence of nitrifying bacteria was indicated in all media but sand and, after 24 days' incubation, these media, with the exception of sand alone, showed strong nitrite reaction.

TABLE IV

COUNTS OF BACTERIA AND FUNGI IN REPRESENTATIVE MEDIA OR MATERIALS SUBSEQUENTLY MIXED WITH SAND

——	Organism	Counts per gram dry weight	
		Before cuttings planted	After cuttings removed
Peat-humus steam-sterilized 2½ hr. at 120° C.	Bacteria	8,000	
	Fungi	82,000	
Peat-humus heated in air blast for 15 hr. at 110° C.	Bacteria	2500	
	Fungi	5000	
Peat-humus–sand, steam-sterilized 2½ hr. at 120° C.	Bacteria	9,590,000	17,600,000
	Fungi	0	160,000
Peat-humus–sand, without sterilization	Bacteria	22,700,000	2,900,000
	Fungi	448,000	120,000
Swedish-peat–sand	Bacteria		4,300,000
	Fungi		21,000
Sand alone	Bacteria		2,000,000
	Fungi		30,000

Effect of Mulched and Mixed Media

Results of the analyses of variance in Table V indicate the manner in which certain rooting responses were affected by media treatments. Data for the statistically significant effects are presented in Table VI. The main feature of the results is the response to active silica. Sand alone was inferior to all

** *Attains 1% level of significance.*

TABLE V

ANALYSIS OF VARIANCE OF RESPONSE OF NORWAY SPRUCE CUTTINGS TO VARIOUS MEDIA UNDER SUBOPTIMUM CONDITIONS

Source of variance	Degrees of freedom	Mean square		
		Number of cuttings		
		Surviving	Rooted	Rooted with new growth
Replicates	3	114.8	256.2	280.8*
Treatments	14	654.2**	596.5**	584.3**
Error	42	137.4	106.0	89.5
Partition of treatments				
Sand versus treatments	1	853.2*	158.9	165.6
Mulching versus mixing	1	311.1	41.8	221.6
Treatments (peats and silica)	6	871.2**	750.3**	809.5**
Treatments × mulching versus mixing	6	461.2**	609.1**	489.4**
Silica versus peats and soil	1	1348.4**	1493.5**	2076.1**

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*

other media, except Swedish peat. On the average, mulching tended to favour survival, have no effect on rooting alone, and have a favourable effect on incidence of both roots and new growth by the same cuttings. However, media showed differential response to mulching and mixing. Mulching with active silica, Swedish peat, and peat–humus tended to be beneficial. Mulches with Petawawa peat had little effect. A sand–soil mixture was superior to a sand–soil mulch.

The highest rooting in the experiment was 52% in sand mulched with active silica. Sand alone produced 12% rooted cuttings. The low average rooting was undoubtedly related to the deliberately imposed suboptimum watering conditions.

Average media effects on number of roots, mean root length, and new growth are shown by the data of Table VII. Mulching sand with peats, soil, and active silica tended to decrease the number and length of roots. However, the mean shoot length of surviving cuttings was greater in mixtures; the effect was reversed for cuttings with both new growth and roots. While differences between media were apparent they were not generally marked. Grinding of Swedish peat decreased root number for cuttings without new growth but had the reverse tendency for cuttings with both roots and new growth; it also favoured root length and length of new growth for such rooted cuttings. Soil media were beneficial to number and length of roots, with the most marked effect on the number of new growth shoots per rooted cutting. Active silica also favoured increased number of roots.

TABLE VI

MEDIA EFFECTS ON NUMBER OF NORWAY SPRUCE CUTTINGS SURVIVING, ROOTED, AND WITH NEW GROWTH AND ROOTED UNDER SUBOPTIMUM CONDITIONS (SEE TABLE V)

Propagation medium	No. of surviving cuttings				No. of rooted cuttings				No. of rooted cuttings with new growth			
	Transformed data		%		Transformed data		%		Transformed data		%	
	Single values	Means	Single values	Means	Single values	Means	Single values	Means	Single values	Means	Single values	Means
Sand	32.6		30		19.5		12		12.8		7	
Mulched with peat humus	55.3	50.6	67	59	36.0	32.3	35	29	32.1	22.5	28	17
Mixed with peat humus	46.0		52		28.6		23		12.0		7	
Mulched with Swedish peat	43.0	35.8	47	35	24.6	16.0	18	11	14.5	7.2	8	4
Mixed with Swedish peat	28.6		23		7.5		3		0		0	
Mulched with ground Swedish peat	41.1	32.2	43	32	15.0	10.8	7	6	11.3	5.6	5	3
Mixed with ground Swedish peat	23.3		20		6.7		5		0		0	
Mulched with Petawawa peat	47.9	51.4	55	61	19.5	28.3	12	24	19.5	20.7	12	15
Mixed with Petawawa peat	54.9		67		37.2		37		21.8		18	
Mulched with ground Petawawa peat	56.3	57.8	68	71	22.1	31.1	18	30	22.1	24.7	18	20
Mixed with ground Petawawa peat	59.4		73		40.1		42		27.3		22	
Mulched with soil	36.1	46.2	35	52	12.9	24.9	7	22	9.1	21.5	5	18
Mixed with soil	56.2		68		36.9		37		33.9		32	
Mulched with active silica	70.5	59.7	85	68	46.0	38.7	52	41	42.0	34.4	45	34
Mixed with active silica	48.9		50		31.3		30		26.9		23	
Necessary difference, 5% level of significance, transformed data only	16.7	11.8			14.7	10.4			13.5	9.5		
Means												
Sand mulched with peats, soil, silica		50.0		57.2		25.2		21.2		21.5		17.3
Sand mixed with peats, soil, silica		45.3		50.5		26.9		25.2		17.5		14.6

PLATE I

FIG. 1. *Representative four-year-old Norway spruce cuttings potted from field March, 1945. (See class of cutting and shading conditions, Part I). Left to right: from small (1½ to 3 in.), from medium (3 to 6 in.), from large (6 to 10 in.) cuttings.*

TABLE VII

AVERAGE EFFECTS OF MEDIA ON THE NUMBER OF ROOTS, MEAN ROOT LENGTH, NEW GROWTH SHOOTS, AND LENGTH OF NEW GROWTH

Media	Rooted cuttings without new growth		Rooted cuttings with new growth				New growth shoots for cuttings without roots [1]	
					New growth shoots			
	No. of roots per rooted cutting	Mean root length, mm.	No. of roots per rooted cutting	Mean root length, mm.	No. per rooted cutting	Mean shoot length, mm.	No. per cutting	Mean shoot length, mm.
Sand	1.7	9	5.0	17	1.2	17	1.3	14
With peat humus	1.4	30	2.3	51	1.2	18	1.4	17
With Swedish peat	1.8	35	2.2	15	1.0	14	1.0	18
With ground Swedish peat	1.4	49	3.0	19	1.0	21	1.0	18
With Petawawa peat	1.6	38	2.3	38	1.1	16	1.4	14
With ground Petawawa peat	2.0	29	2.2	41	1.2	19	1.3	19
With soil	2.0	45	3.0	45	1.7	18	1.3	16
With active silica	2.3	33	3.1	35	1.3	15	1.4	17
Means for media								
Mulched with peats, soil, and silica	1.4	30	2.5	32	1.2	19	1.3	18
Mixed with peats, soil, and silica	2.0	43	2.8	45	1.3	14	1.3	25

Development of new growth by rooted cuttings appeared to be associated with increase in number of roots, and the mean root length was favourably affected in sand, peat-humus, and ground Petawawa peat; root length was reduced for cuttings with new growth in Swedish peat. However, neither number of shoots nor mean shoot length appeared to be appreciably affected by the presence or absence of roots. The favourable effects of soil were confined to number of shoots for rooted cuttings only.

Petawawa peat was substantially similar in all respects to peat-humus.

DISCUSSION

Effects of size of cutting on rooting have been pronounced; on the average, 96% of the cuttings from 3 to 6 in. long rooted. The 71% rooting shown by cuttings 6 to 10 in. long is possibly as interesting from the tree breeders' point of view. Fig. 1 shows the relative growth attained by a representative cutting of each of the three length classes studied. These results suggest that use of larger cuttings should speed up the operations of the breeder. It is too early to finally conclude that these large cuttings are less prone towards plagiotropic growth forms, though this is indicated.

Greenhouse propagation of Norway spruce cuttings in a peat-humus–sand medium resulted in rooting of the order of 80%. The advantageous features of domestic peat humus are as marked as they were under outdoor propagation conditions (3-6, 20). However, the weekly application of solutions of potassium acid phosphate and magnesium sulphate to a Swedish-peat–sand medium resulted in 96% rooting. It has already been suggested that available mineral nutrients are among the factors responsible for the desirable effects of peat

humus; these results confirm this conclusion (6, 17). However, cuttings planted in sand with a ½ in. mulching layer of peat humus gave results fully as good as occurred in the mixture. Since the amounts of humus, and consequently available nutrients, differ widely under these conditions, other factors would seem to be involved.

Steam sterilization of peat humus failed to affect rooting therein while heating in an air blast at 110° C. had a detrimental effect. The dry heat may have irreversibly affected the colloidal system. Inactivation of trace constituents seems unlikely since extraction with methyl alcohol failed to affect the response of peat humus and addition of the dried alcoholic extract to the Swedish peat medium had no effect. Extraction with 5% acetic acid had no effect though sodium hydroxide extraction was markedly injurious; the latter probably related to changes in the physical state of the medium.

Under suboptimum conditions the responses of cuttings to mulching or mixing of peats, soil, and active silica with sand varied. Soil mixtures were much better than soil mulches; active silica was much superior as a mulch and peat humus was substantially similar in both the mulched and mixed condition. The superior results from active silica call for special comment. Since results with this material, lacking mineral nutrient, surpassed those with peat humus, it may be concluded that the physical nature of the medium is of outstanding importance under suboptimum propagation conditions. It would be interesting to compare active silica and peat humus media under the most favourable conditions. Until this is done, it may be concluded that a combination of physical factors such as moisture retention and availability of inorganic nutrients are important considerations in selection of media for propagation of conifer cuttings.

Within a fairly narrow pH range from a little below 7 to above 8, the cuttings showed better rooting at lower, rather than higher pH levels.

Part II. Effects of phytohormone chemicals, nutrient salts, thiamin, and nicotinic acid

Cuttings were prepared from branches of the collection described under "Class of cutting and shading conditions," Part I. All chemical treatments were applied in a talc carrier dust. Preparation of dusts and methods of treating cuttings have been described (7, 10).

Naphthyl Chemicals and Indolylbutyric Acid at Four Equivalent Concentrations

Cuttings 3 to 6 in. long were treated with talc dusts of a factorial series of 16 preparations involving four growth stimulating chemicals each at four concentrations (15). Untreated and talc treated control groups were added. Naphthylacetic, naphthylbutyric, and indolylbutyric acids and the potassium salt of naphthylhexoic acid (13, 14, 18) were used in equimolecular quantities based on 4000, 2000, 1000, and 500 parts of naphthylacetic acid per million parts of talc dust. Cuttings were stored under a damp cloth for not more than two hours between preparation and treatment. There were 10 cuttings

to a group and four complete replicates of the 18 treatments. Cuttings were planted Dec. 17, 1940, and removed for examination May 8, 1941.

Application of Naphthylbutyric Acid, Nutrient Salts, Thiamin, and Nicotinic Acid

Freshly prepared moist cuttings from 1½ to 3 in. long were dust treated and planted immediately. Cuttings were treated with the following talc dusts: (*a*) talc alone; (*b*) talc containing 1000 p.p.m. of naphthylbutyric acid; (*c*) talc containing 1% of nutrient salts; (*d*) talc containing 1000 p.p.m. napthylbutyric acid and 1% of nutrient salts; (*e*) the four dusts, *a* to *d*, to each of which was added 50 p.p.m. of thiamin + 50 p.p.m. of nicotinic acid; and (*f*) the four dusts, *a* to *d*, to each of which was added 100 p.p.m. of thiamin alone. This provided a factorial series of 12 treatments. Hoagland's mixture of nutrient salts was used (9, 14, 18, 23). Four additional groups included untreated cuttings, cuttings treated with a finely ground charcoal dust, and with charcoal dust containing 1000 and 2000 p.p.m. of naphthylbutyric acid. The total of 16 treatments involved groups of 10 cuttings with four complete replications. Cuttings were planted Dec. 19, 1940, and removed for examination Apr. 2, 1941.

Results

Effects of Naphthyl Chemicals and Indolylbutyric Acid at Four Equivalent Concentrations

In Table VIII are given only the average effects of chemicals over the four concentrations and concentrations over all four chemicals, since there were no significant differential effects involved. Naphthylacetic acid treatment had markedly injurious effects and indolylbutyric acid was markedly superior to naphthylbutyric acid in respect of both number of rooted cuttings, and number with new growth, but only slightly superior to the potassium salt of naphthylhexoic acid. The injurious effects of these chemicals were marked at the 4000 p.p.m. level. None of the other three dosages differed in respect of number of cuttings rooted but the 500 p.p.m. level was superior to all others in the number of cuttings showing new growth. The average rooting of talc treated and untreated cuttings exceeded 80%, a value substantially equal to that obtained with indolylbutyric acid. The high level of rooting is attributed to the presence of peat humus in the propagating medium.

Effects of Naphthylbutyric Acid, Nutrient Salts, Thiamin, and Nicotinic Acid

Table IX shows the results of statistical treatment of the data. Significant differences existed between untreated, charcoal treated, and talc treated cuttings in the number of cuttings rooted and the number of roots per rooted cutting. Mean root length was affected by the vitamin treatment and also by the interaction of naphthylbutyric acid and nutrient. Naphthylbutyric acid in a charcoal carrier had a marked effect on the number of rooted cuttings.

The average effects of all talc and charcoal treatments are compared in Table X with the results for untreated groups and for those with only talc and charcoal; data for vitamin effects also are given. It is apparent that

TABLE VIII

AVERAGE EFFECTS OF HORMONE CHEMICALS ON THE ROOTING AND NEW GROWTH OF NORWAY SPRUCE CUTTINGS

Response	Controls		Naphthyl-acetic acid	Naphthyl-butyric acid	Potassium salt of naphthyl-hexoic acid	Indolyl-butyric acid	Concentration of chemicals, p.p.m.				Necessary difference, 5% level
	Un-treated	Talc treated					4000	2000	1000	500	
Number of rooted cuttings											
Transformed data	75.6	68.9	44.2	57.5	59.6	65.2	44.6	62.1	57.3	62.7	8.1
%	87	82	47	68	72	82	49	73	68	78	—
Number of cuttings with new growth											
Transformed data	63.4	76.2	43.0	52.8	58.4	61.9	47.0	52.3	54.1	62.8	8.4
%	75	93	47	61	71	75	52	63	64	76	—

TABLE IX

ANALYSIS OF VARIANCE OF RESPONSE OF NORWAY SPRUCE CUTTINGS TO DUST TREATMENTS WITH PLANT HORMONE, VITAMIN, AND NUTRIENT CHEMICALS IN TALC AND CHARCOAL

Source of variance	Degrees of freedom	Mean square			
		No. of cuttings rooted	No. of cuttings with new growth	No. of roots per rooted cutting	Mean root length
Replicates	3	88.8	230.4	0.138	131.4
Untreated vs. charcoal vs. talc	2	374.7*	87.3	1.132*	9.6
Naphthylbutyric in charcoal	2	587.4*	255.9	0.251	51.7
Talc treatments					
Naphthylbutyric	1	332.9	170.3	0.533	45.1
Nutrient	1	190.4	354.3	0.298	200.5
Vitamin	2	50.7	37.0	0.142	301.0**
Naphthylbutyric × nutrient	1	29.4	5.1	0.156	290.6*
Naphthylbutyric × vitamin	2	127.3	174.9	0.048	15.4
Nutrient × vitamin	2	281.9	73.5	0.480	27.1
Naphthylbutyric × nutrient × vitamin	2	69.1	14.9	0.353	12.6
Error	45	115.1	147.6	0.350	53.6

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*

TABLE X

AVERAGE EFFECT OF DUST TREATMENT AND VITAMIN ON ROOTING OF NORWAY SPRUCE CUTTINGS

Treatment	No. of rooted cuttings		No. of roots per cutting	Mean root length, mm.
	Transformed data	%		
Untreated	81.7	93	2.6	
Talc alone	76.2	93	3.2	
Charcoal alone	62.3	78	3.0	
Average of all:				
Talc treatments	68.1	81	2.9	
Charcoal treatments	71.7	87	3.3	
Talc treatments without thiamin or nicotinic acid				34.7
Talc treatments with 50 p.p.m. of each of thiamin and nicotinic acid				43.3
Talc treatments with 100 p.p.m. of thiamin				38.0
Necessary difference, 5% level				5.2

charcoal tended to reduce rooting. The number of roots per rooted cutting was lowest for the untreated cuttings. The combined treatment of 50 p.p.m. each of thiamin and nicotinic acid increased mean root length. Since 100 p.p.m. thiamin was without significant effect, nicotinic acid probably was the stimulating factor.

The effects of naphthylbutyric acid in charcoal on rooting are described by the data of Table XI. The 2000 p.p.m. dosage was significantly above both the control and intermediate level, effecting 98% rooting.

TABLE XI

EFFECTS OF NAPHTHYLBUTYRIC ACID IN CHARCOAL ON ROOTING OF NORWAY SPRUCE CUTTINGS

Kind of data	Charcoal only	Naphthylbutyric acid in charcoal, p.p.m.		Necessary difference, 5% level
		1000	2000	
Transformed	62.3	67.5	85.4	15.3
%	78	85	98	

The nature of the interaction effects of naphthylbutyric acid and nutrient treatments with respect to mean root length are described by the data of Table XII. In the absence of nutrient salts, naphthylbutyric acid, on the average, tended to reduce root length. In the absence of naphthylbutyric acid, nutrient salts had a similar reducing effect. When combined chemical treatments were used the depressive tendency was avoided.

TABLE XII

INTERACTION EFFECTS OF NAPHTHYLBUTYRIC ACID AND NUTRIENT TREATMENT ON THE MEAN ROOT LENGTH OF NORWAY SPRUCE CUTTINGS, MM.

Nutrient concentration in talc, %	Naphthylbutyric acid in talc, p.p.m.	
	0	1000
0	42.2	39.2
1	32.2	40.2

Necessary difference, 5% level = 6.0

DISCUSSION

The injurious effects of plant hormone chemicals in a talc carrier are in agreement with previous results for propagation of conifer cuttings (9, 16, 19, 21). While naphthylbutyric acid and the potassium salt of naphthylhexoic acid were less injurious than naphthylacetic acid, the effects were substantially

similar. It was somewhat surprising to find that indolylbutyric acid failed, on the average, to show either injury or stimulation. The progressive increase of injury with ascending concentration of these chemicals agrees with the results of earlier experiments on plant hormone chemicals (18).

In the preliminary work with naphthylbutyric acid in a charcoal carrier the 98% rooting obtained was the highest percentage reached in greenhouse propagation experiments carried on by the authors.

While nutrient salt treatment tended to reduce root length, this effect was not apparent when nutrient salts were applied along with naphthylbutyric acid. Vitamin treatments were generally ineffective although an increase in mean root length was observed when both thiamin and nicotinic acid were present.

The high average level of rooting attained in these experiments was largely related to the peat-humus–sand propagation medium. Favourable effects of this medium have been shown by results from both greenhouse and outdoor propagation (3-6, 20).

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STUDIES ON THE *FUSARIUM* OF MUSKMELON WILT

II. INFECTION STUDIES CONCERNING THE HOST RANGE OF THE ORGANISM AND THE EFFECT OF ENVIRONMENT ON DISEASE INCIDENCE[1]

By John J. Miller[2]

Abstract

The effect of environment on disease incidence was investigated in some detail. A comparison of disease incidence resulting from equal degrees of infestation of sterilized and unsterilized soils led to the conclusion that the factor responsible for the suppression of disease incidence in unsterilized soil was biological. Temperature studies indicated a definite decrease in disease incidence above 30° C. but no minimum temperature was found below which the host grew well and escaped infection. Low soil moistures appeared to accentuate disease incidence. Seedlings grown in naturally infested soil seldom wilted and since the pathogen was shown to be present in this soil, a protective effect must have been operating. This was found associated with a severe stunting and both appeared due to biological causes. Evidence indicated that the wilt *Fusarium* was not important with regard to the stunting. Cultural variants of the pathogen were found less virulent than the wild type on all host varieties tested, emphasizing the importance of employing the highly virulent wild type strain when infesting soils for resistance trials.

Introduction

The present paper is the continuation of a series that began with the cultural studies on the pathogen described under Part I (6) and involves an investigation of the pathogenic behaviour of the organism. Since the experiments outlined here were carried on simultaneously with those of Part I, they were influenced by the more basic cultural studies. This is apparent in the later experiments where the importance of maintaining the purity of the wild type was fully appreciated. It also led to a search for differential pathogenicity among certain cultural variants of the organism and this provided further evidence for the validity of the 'wild type' concept.

The taxonomy of the organism has been discussed in Part I and the conclusion was reached that it is closely similar to a pathogen of muskmelons described by Leach and Currence (4) as *Fusarium bulbigenum* Cke. and Mass. var *niveum* Wr. f. 2.

Materials and Methods

In most respects materials and methods remained constant during the following experiments and were similar to those employed under Part I (6). In the earlier experiments oat-hull medium was employed as inoculum. This was prepared by moistening ground oat hulls with a 1% dextrose solution in the proportion of 5 : 1 by volume. Cornmeal–sand medium was later

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[2] *At the time, Class Assistant, Department of Botany, University of Toronto; now Agricultural Assistant, Dominion Laboratory of Plant Pathology, Harrow, Ont.*

employed since it was more readily prepared. Further, to evade a severe seed rot caused by other organisms, in unsterilized soil, a method of seed treatment was developed and adopted as standard procedure.

Experiments

(*a*) *Comparison of Disease Incidence in Various Soils*

The soils employed were: *A*—a mixture composed of ½ loam, ¼ sand, ¼ leaf mould; *B*—Vineland Clay Loam; *C*—light sandy soil from one of the sandy knolls that appear irregularly throughout the Vineland Clay Loam area (this will be referred to as 'sandy knoll' soil); *D*—Vineland Fine Sandy Loam.

This experiment was performed in the University of Toronto greenhouses during the fall of 1942. Six flats of each soil type were employed, three of which were sterilized. Oat-hull inoculum at the rate of 2% by volume was added to each of these except the two check flats to which sterilized medium was added.

The disease incidence has been calculated on the basis of the number of seeds that came up. Emergence values of from 58 to 90% were obtained in the inoculated flats and from 55 to 78% in the checks. The emergence was consistently less in the unsterilized soils. Daily records were kept of the number of seedlings that wilted and from these data the wilt progress curves in Fig. 1 were calculated. It is evident that disease incidence tended to be more severe in sterilized soils. This may be rationalized on the assumption

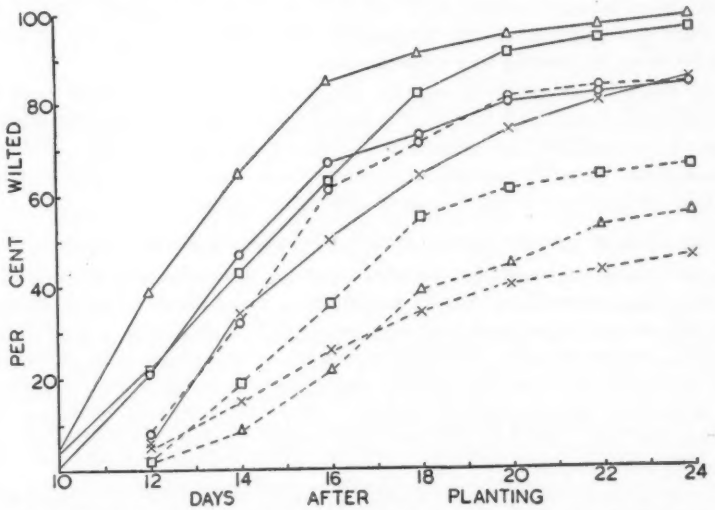


FIG. 1. *Comparison of disease incidence in sterilized and non-sterile samples of four soil types, infested with the muskmelon wilt Fusarium.* ○ = *Leaf mould compost.* □ = *Vineland Clay Loam.* △ = *'Sandy knoll' soil.* × = *Vineland Fine Sandy Loam.* ——— *Sterilized soil.* - - - - - - *Non-sterile soil.*

that the organisms that predominated in the unsterilized soils were less favourable to the pathogen than those prevailing in the sterilized soils. A possible exception to this was the leaf-mould compost where there seemed to be little difference after about 17 days.

If the physical nature of the soil were the decisive factor affecting disease incidence one would have expected the 'sandy knoll' soil and the Vineland Fine Sandy Loam to give similar curves since these are both sandy types. Yet wilt progress curves in these two soils in the sterilized state differ widely. In fact, the graphs for 'sandy knoll' soil and Vineland Clay Loam converge toward the end of the experiment. The same is true of the leaf mould compost and the Vineland Fine Sandy Loam. Hence it may be concluded that no correlation is evident between soil type and disease incidence.

It is not likely that the differences observed were due to predisposition of the host in unfavourable soil. Muskmelons prefer a loose sandy soil like the 'sandy knoll' soil, yet the highest disease incidence in sterilized soil was found in this type. Of the other three soils, the Vineland Clay Loam showed the next greatest disease incidence to the 'sandy knoll' soil. Since heavy clay soil is not favourable to melons, the comparison suggests that the physical nature of the soil was not responsible for the variation in disease incidence.

The organism was recovered from some of the wilted seedlings but not as the original wild type described in the first paper of this series (6). Eight isolations yielded seven cultures classified as dark semiraised and one intermediate appressed. It is thus likely that the oat hulls were inoculated with a mixture of mutant strains that had crowded out the parent. Since the inoculum was added uniformly to the flats, the value of the experiment is not considered to be lessened as a consequence.

The greatest difference in disease incidence between sterilized and unsterilized soils occurred in the 'sandy knoll' type. After 20 days the amount of wilt in unsterilized soil was only half that found in sterilized. This soil was employed in further experiments since it was easily worked with and was available in quantity at the Vineland Experiment Station.

(*b*) *The Effect of Various Degrees of Soil Infestation on Disease Incidence*

A dilution series involving two strains of the organism was performed in sterilized and unsterilized 'sandy knoll' soil. The following concentrations of cornmeal–sand inoculum were employed: 0, 0.1, 0.5, 1.0, 2.0, 4.0% of the total volume of soil. Flasks of cornmeal–sand medium were inoculated with mycelium from freshly isolated single spore cultures of both wild type and a derived light appressed mutant. After 18 days this inoculum was incorporated into the soil. In each flat, which contained 10 litres of soil, 140 seeds were planted.

The experiment was first set up in July, 1943. An effort to approximate field conditions as closely as possible led to the failure of the first planting. Before the seedlings emerged, the flats were wet by a heavy rain and severe seed rot ensued.

Before replanting, some methods of seed treatment were tested in the hope of evading the rot. The following treatment was found to be very effective. The seed was soaked for three hours in tap water and then spread out on papers until the surface was dry. This was followed by shaking the seeds in a container with enough Semesan dust to cover them heavily, after which they were planted. Subsequently whenever an experiment involved unsterilized soil, this procedure was followed.

The flats were thus replanted and kept in the greenhouse for the duration of the experiment. Emergence values of from 72 to 87% were obtained. The data are summarized in Fig. 2, which compares the disease incidence in terms of percentage of wilt after 20 days.

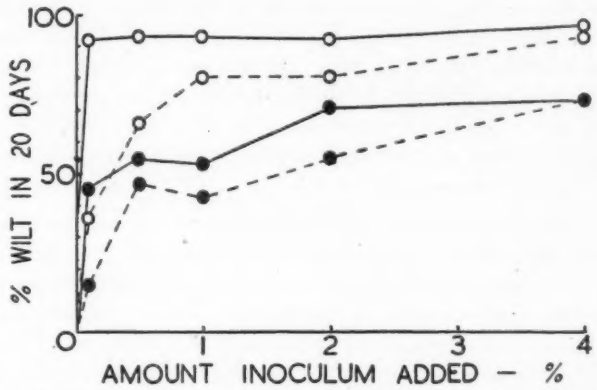


FIG. 2. *Disease incidence after 20 days in sterilized and non-sterile 'sandy knoll' soil infested in various degrees with inoculum of the wild type and a light appressed mutant culture.* ○ = *Wild type.* ● = *Light appressed.* ———— *Sterilized soil.* - - - - - - *Non-sterile soil.*

The depressing effect on disease incidence of the competitive factor was most evident where the parasite was added at the greater dilutions. Both strains of the parasite were affected in this way. This observation suggests that the parasite is hindered in establishing itself in unsterilized soil, perhaps owing to the competition of other organisms that may slow down the spread of the primary inoculum. Disease incidence was not increased greatly by concentrations of inoculum higher than 1 to 2%.

The fact that even a low level of soil infestation is sufficient to cause severe wilt is of some significance in regard to the epidemiology of the organism. It suggests that fields may become severely infested from the introduction of small quantities of inoculum and that the survival of even a small amount of inoculum from one season to the next would suffice to maintain the parasite after it became established.

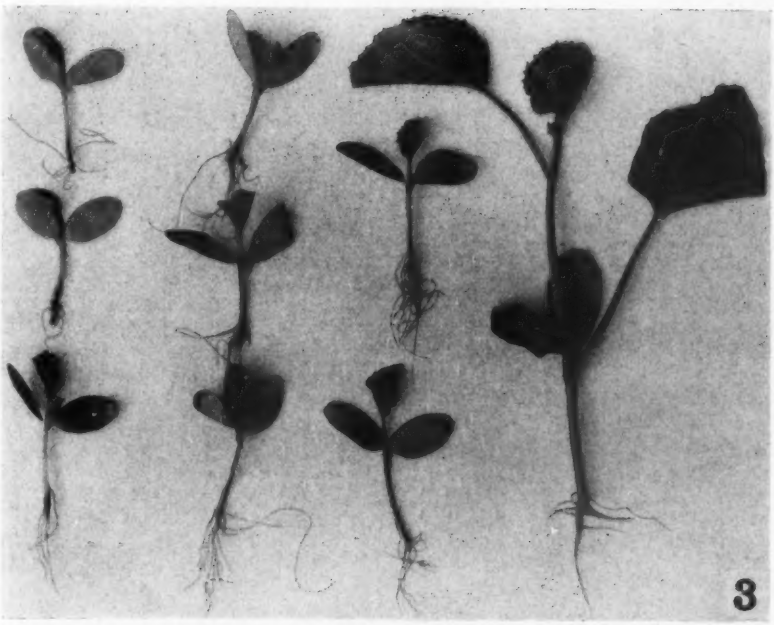
(c) *Experiments Dealing With the Suppression of Seedling Wilt in Naturally Infested Soil*

During the summers of 1942 and 1943 naturally infested soil was obtained from four melon fields.

It was found that seedlings grown in flats of this soil seldom wilted. In one instance only one seedling among 124 had wilted after a month although, as will be evident in the dilution experiment, the pathogen must have been present in this soil. It is thus evident that the host is protected in some manner. Associated with this protective effect, however, was a marked stunting (Fig. 3). In the case just mentioned, after a month of growth the fresh weight of the seedlings in a flat of naturally infested soil averaged only 1/6 that of the seedlings in a sterilized soil check. Toward the end of this period the stunted seedlings became chlorotic, first in the cotyledons and later in the secondary leaves as well. Another symptom was the rotting of the primary root, as a result of which adventitious roots developed in an unusually abundant manner from the lower region of hypocotyl. A yellowish discoloration was found at the base of the hypocotyl and occasionally a lesion extended for a short distance up the side.

Although the wilt *Fusarium* has been isolated from mature plants that wilted in the melon fields and from the few seedlings that showed typical wilt symptoms in naturally infested soil, it was found very difficult to isolate it from non-wilted seedlings growing in this soil. Most attempts at isolation yielded either a *Penicillium* sp. or an unidentified organism. Experiments with the latter showed it to be mildly pathogenic on muskmelon seedlings when incorporated into sterilized soil in a concentration of 1% by volume. Under these conditions it produced conspicuous lesions at the base of the hypocotyl but no stunting. Small yellowish lesions were also observed on the cotyledons. These appeared 8 to 10 days after emergence and it seems likely that the cotyledons became inoculated as they emerged, since the organism could not be isolated from the upper part of the hypocotyl although it was readily isolated from the leaf lesions. These lesions did not appear on the secondary leaves. The organism appears very sensitive to the competitive action of other soil organisms since, when this soil was replanted, no lesions appeared on the cotyledons, although there was an occasional lesion near the base of the hypocotyl, indicating that in the interval between the two plantings the competition factor had become more effective. When this soil was mixed in equal proportions with sterilized soil containing inoculum of the wilt *Fusarium* no protection against seedling wilt was observed, nor was any stunting produced.

The experimental work that follows represents an effort to elucidate the nature of this protective effect and to determine its relation to the stunting.



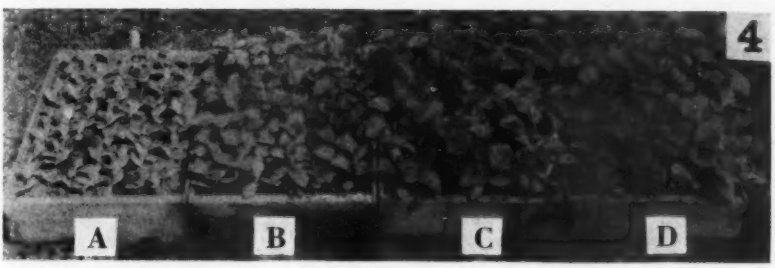
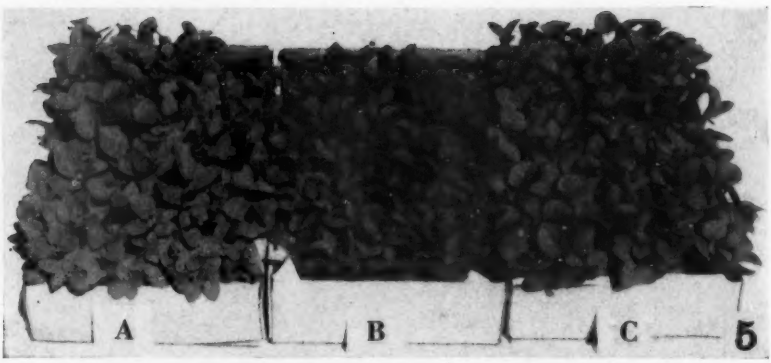


FIG. 3. *Stunting of seedlings grown for one month in a flat of naturally infested soil. The seedling to the right was grown in sterilized soil for the same period.*

FIG. 4. *The effect of diluting naturally infested soil in various proportions with sterilized 'sandy knoll' soil. A. Undiluted naturally infested soil. Note chlorosis and severe stunting. B. Fifty per cent naturally infested soil. C. Twenty-five per cent naturally infested soil. D. Sterilized 'sandy knoll' soil.*



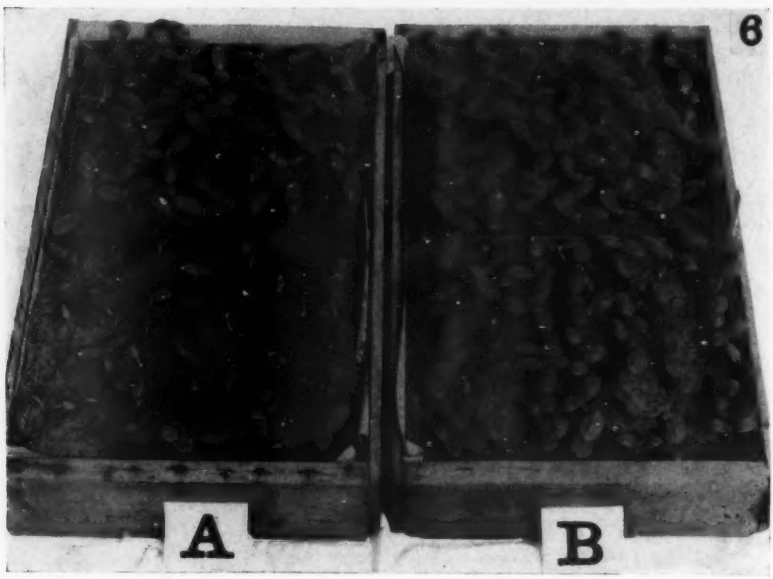


FIG. 5. *The effect of soil sterilization on the stunting of melon seedlings in naturally infested soil. A. Steam sterilized. B. Unsterilized. C. Chloropicrin sterilized.*

FIG. 6. *The effect of treating melon seeds with Semesan dust on disease incidence in heavily infested soil. The seeds in the far half of each flat were treated and those nearest the camera were untreated. A. Infested soil. B. Check. Non-infested soil.*

(i) The Effect of Adding Inoculum of the Wilt Fusarium to Naturally Infested Soil

This experiment was performed to determine the extent to which wilt could be induced in naturally infested soil by artificially increasing the concentration of *Fusarium*.

To each of six pots two litres of naturally infested soil were added and three of these were steam sterilized. Two of each were then inoculated with a 1% concentration of cornmeal–sand medium on which the wild type strain was growing. Each pot was planted with 30 seeds. The results are summarized in Table I.

TABLE I

THE EFFECT OF ADDING 1% OF INOCULUM TO NATURALLY INFESTED SOIL

Soil	Emergence, %	Wilt in 24 days, %	Stunting
Sterilized,			
Not inoculated	90	7.4	Absent
Inoculated	88	98	Absent
Unsterilized,			
Not inoculated	83	0	Severe
Inoculated	70	7.1	Severe

The addition of inoculum of *Fusarium* to unsterilized soil caused a slight increase in the amount of wilt but the value obtained was unusually low, even for unsterilized soil. Since wilt was severe in this soil when it was sterilized previous to inoculation, the indication is that the action of other organisms present in naturally infested soil was responsible for the protection of the host. No stunting was observed in the sterilized soil but it was severe in unsterilized soil.

(ii) The Effect of Dilution With Sterilized Soil

Naturally infested soil was diluted with freshly sterilized 'sandy knoll' soil in a series of eight concentrations and in each flat 140 seeds were planted. A record was kept of the number of plants that wilted and a month later the average weight of the seedlings in each flat was determined. These results are shown in Table II.

Although an average of 108 seedlings emerged in each flat, it is apparent from the table that very few of these wilted. It is remarkable that the amount of wilt was greatest at the higher dilutions. For example, while only one seedling wilted in the undiluted soil, when it was diluted to 1% by volume with sterilized soil, 13 seedlings wilted in the same time, and, at a dilution of 0.1%, 11 seedlings wilted. The wild type *Fusarium* was readily isolated from these wilted seedlings, whereas from a total of 64 non-wilted seedlings it was isolated in only one instance, which occurred in the 25% flat.

The plants in all the dilutions of naturally infested soil showed a tendency to produce adventitious roots from the lower part of the hypocotyl although

TABLE II

THE EFFECT OF DILUTING NATURALLY INFESTED SOIL WITH FRESHLY STERILIZED 'SANDY KNOLL' SOIL

Concentration of naturally infested soil, %	Number of seedlings wilted	Average weight of seedlings, gm.	Percentage of check weight
100	1	0.42	18
50	1	1.15	48
25	4	1.68	71
10	7	1.74	73
5	9	1.99	84
1	13	1.70	71
0.1	11	1.62	68
0	1	2.38	100

this was only slightly apparent at 0.1%. The discoloration of the base of the hypocotyl was most marked in undiluted naturally infested soil and became less and less evident until at 1% only a few plants showed it.

The isolations demonstrated the organism to be present in the soil and since it caused pronounced seedling wilt only when greatly diluted with sterilized soil, a protective effect must have been operating. This may be due to a complex of organisms, which is disturbed by dilution. The addition of freshly sterilized soil provides a supply of nutrient and those organisms best able to take advantage of it would tend to predominate for a time, at least. The great increase of wilt at the higher dilutions indicates that the wilt *Fusarium* is able to profit from the new situation.

The extreme degree of stunting noted in naturally infested soil was largely overcome by dilution (Fig. 4). Thus, this effect may also be due to a complex of organisms that can be disturbed by dilution. It remains to be determined whether the organisms responsible for the protective effect are also involved in the stunting.

(*iii*) *The Effect of Soil Sterilization on the Stunting of Seedlings*

In a preceding experiment it has been shown that stunting in naturally infested soil could be avoided by steam sterilization, indicating that the effect was due to organisms present in the soil rather than to the composition of the soil itself. Since the possibility remained that such a drastic method of soil sterilization would alter the physical or chemical properties of the soil sufficiently to account for this, a partial check was obtained by sterilizing with chloropicrin.

Enough naturally infested soil to make three flats was mixed thoroughly. One-third was then steam sterilized and another third was sterilized with chloropicrin. Chloropicrin sterilization was accomplished by keeping the soil for three days in a tight container with the addition of 1 cc. chloropicrin to each 3 litres of soil. This sterilization was not quite complete since by plating out some of the soil immediately after opening the container, a bacterial

organism was obtained which, incidentally, proved to be antagonistic to the wilt *Fusarium* in culture. The steam sterilization was found to be complete. Ten days were allowed for the chloropicrin to disappear from the soil and all three flats were then planted with 140 seeds each.

Within 15 days after planting, the seedlings growing in unsterilized soil were noticeably stunted (Fig. 5). There was no evidence of stunting in either of the sterilized flats and the seedlings were quite as well developed as those in a flat of sterilized 'sandy knoll' soil that was planted on the same date.

Stunting was thus avoided by both types of sterilization. Since chloropicrin left at least one organism viable in the soil, it must have been a less drastic form of treatment than steaming under pressure. This was further evident in the texture of the soil since after steaming the soil particles tended to adhere in larger lumps and the ability to absorb water appeared lessened. But chloropicrin caused no evident deviation from the properties of unsterilized soil.

These results suggest that the unfavourable factor involved here is not inherent in the chemical or physical nature of the soil environment and is probably biological.

(*iv*) *Stunting in Hosts Resistant to the Muskmelon Fusarium*

The Mango Melon or Vine-Peach has been shown in resistance trials to be highly resistant to the wilt *Fusarium*. Yet it was found that this host became stunted in naturally infested soil to about the same degree as the susceptible variety Bender's Surprise. A further experiment showed that cucumber seedlings of the variety Straight 8 were likewise affected but not, apparently, as severely as muskmelons.

Since the range of varieties affected by stunting is broader than in the case of wilt, the indication is that other organisms than the muskmelon wilt *Fusarium* are involved here. In fact, this *Fusarium* may be of little or no importance with regard to the stunting.

It has been pointed out that a mildly pathogenic organism was isolated from stunted seedlings. This alone was unable to cause stunting but it is possible that the operation of a number of such pathogens would suffice to produce the effect observed. The facts that stunting was prevented by soil sterilization and partly avoided by dilution lend further support to this view.

(*d*) *The Effect of Soil Temperature on Disease Incidence*

During the course of this work six experiments were performed in the Wisconsin temperature tanks in the University of Toronto greenhouses. The first two were considered to be only preliminary since during the course of these a standardized technique was developed and adopted for the succeeding four. The six tanks available were adjusted to temperatures of 18°, 21,° 24°, 27°, 30°, 33° C. An additional variable, soil moisture, was introduced into the first two experiments to be considered while in the final two, six strains of the organism were involved.

The soil employed in these experiments was a leaf-mould compost containing ½ loam, ¼ sand, ¼ leaf mould. For the first four experiments inoculum was introduced into the sterilized compost in the proportion of 2% by volume of oat-hull medium that had been inoculated with a culture of the organism 30 days previously. The inoculum here was prepared before the importance of maintaining cultural purity was fully appreciated. It is likely that the original wild type strain as described in the first paper of this series (6) was not present in the soil since numerous isolations from wilted plants yielded only mutants that were chiefly of the dark semiraised type. Before the soil was placed in the cans, water was added to obtain three moisture levels, which were maintained during the experiments by periodic weighings.

In the first experiment to be considered, 60 seeds were planted in each can at a depth of 2 cm. Each temperature tank provided accommodation for eight cans. Two of these were filled with infested soil at each of the lower moisture levels while one contained soil at the highest soil moisture and the remaining three cans, one at each of the three soil moistures, served as checks. Moisture determinations made at the beginning of the experiment showed that the moisture levels were 27, 36, and 45% of the moisture holding capacity of the soil. The air temperature of the greenhouse throughout this experiment and the one to follow varied from 66° to 95° F. but on warm days it sometimes rose to 100° F. The humidity varied from 90 to 95% of saturation at night to as low as 10 to 20% on bright days. Efforts were made to maintain the humidity as high as possible by sprinkling water on the floor. The first wilt symptoms were observed nine days after planting at 24°, 27°, and 30° C. The disease incidence after 20 days under all combinations of temperature and moisture is summarized in Fig. 7.

At the highest soil moisture employed, disease incidence was found to be greatest at the lower soil temperatures. Although no marked effect of soil

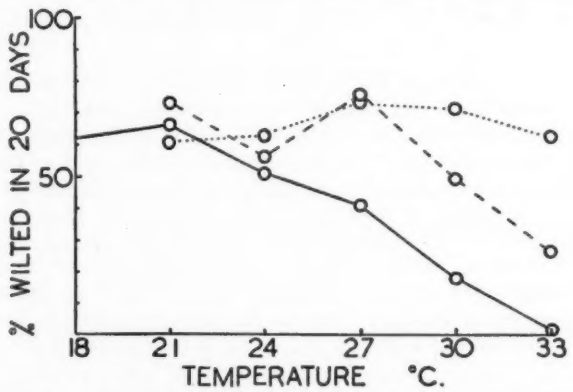


FIG. 7. *Disease incidence in infested soil under varying conditions of soil temperature and soil moisture.* ———— *High soil moisture.* - - - - - *Medium soil moisture.* ..... *Low soil moisture.*

moisture was evident at the lower temperatures, toward the upper end of the temperature scale the disease incidence was definitely increased at the lower soil moistures. The host was found to show the best development at the higher soil temperatures as shown in Fig. 8. From this graph it is also evident that high soil moisture favoured the growth of the host. The optimum temperature for radial growth of the parasite in culture on plates of potato dextrose agar was from 24° to 30° C. with a marked decrease on either side of this range.

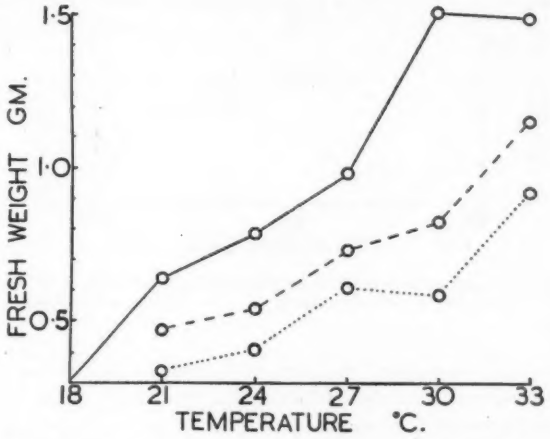


FIG. 8. *Host development in non-infested soil under varying conditions of soil temperature and soil moisture.* ———— *High soil moisture.* - - - - - *Medium soil moisture.* ····· *Low soil moisture.*

No correlation between disease incidence and the growth of the parasite is evident from these data but, on the other hand, disease incidence shows an inverse correlation with the optimum conditions for the growth of the host. It is likely, therefore, that high disease incidence at low temperatures was due to a predisposition of the host. At the higher soil temperatures a predisposition due to low soil moisture seems evident.

The next experiment was performed with the same soil but to avoid the low emergence values encountered when seeds are planted at the lower soil moistures, the seeds were first planted in sterilized soil in 2-in. pots and after 17 days eight of these were transferred to each can of the temperature tanks. In all other respects the technique followed was that of the preceding experiment.

Here also, as shown in Fig. 9, disease incidence increased toward the lower end of the temperature range. However, no increase of disease incidence due to low soil moisture was evident in this case although here, also, the growth of the host was found to be favoured by high soil moisture. Thus it seems likely that the predisposing effect of low soil moisture operates best in the

early seedling stages and may be avoided by planting seedlings a few weeks old. This conclusion is of some significance with regard to the field pathology of the disease. Since the soils best suited to melons are of a light, sandy, and

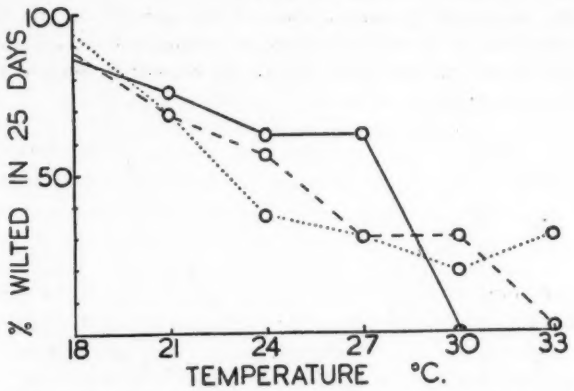


FIG. 9. *Disease incidence in infested soil under varying conditions of soil temperature and soil moisture.* ———— *High soil moisture.* - - - - - *Medium soil moisture.* ····· *Low soil moisture.*

well-drained type, it is evident that the predisposing effect of low soil moisture on seeds germinating in infested fields might be serious. But it is the usual practice among growers to plant the seeds first in sods or in pots and these are later transferred to the field. Thus the cultural practices employed commercially in this respect probably aid in avoiding severe disease incidence.

Two further experiments were performed during October, November, and December, 1943, to investigate the temperature relations of several strains of the organism. The soil of the preceding experiments served also for these but it was first re-sterilized and then inoculated in the proportion of 1% by volume with cornmeal–sand medium on which the following six strains of the organism had grown for seven days: the wild type, the dark semiraised, intermediate appressed, light appressed, very light appressed, and one of the more virulent of the ultra-violet mutants obtained during the irradiation experiments described in Part I of the present studies (6). In addition to the six inoculated cans, each tank contained two cans of sterilized soil, which served as checks. Melon seedlings that had been planted in 2-in. pots three weeks previously were then transplanted into the cans. Each ball of soil thus transferred contained two seedlings and the total number of seedlings per can was 16. After the conclusion of the first of these experiments, the soil in the cans inoculated with each strain was mixed thoroughly to equalize the degree of infestation and replaced in the cans. The soil moisture throughout these two experiments was approximately 40% of the moisture holding capacity and was kept uniform, as in those previously described, by periodic additions of water to bring the cans back to their original weight. The air

temperature of the greenhouse varied from 70° F. to 92° F. and the relative humidity from 95 to 50% of saturation but it sometimes fell much below this on warm days. Water was frequently sprinkled about in the greenhouse to maintain the humidity as high as possible.

The disease incidence in these two experiments 20 days after transplanting is shown in Figs. 10 and 11. Considering first Fig. 10, it is apparent that in this experiment the wild type showed the greatest virulence of all six strains throughout the temperature range. The ultra-violet mutant and the dark semiraised followed in order of virulence. The least virulent of all, as usual, was the very light appressed.

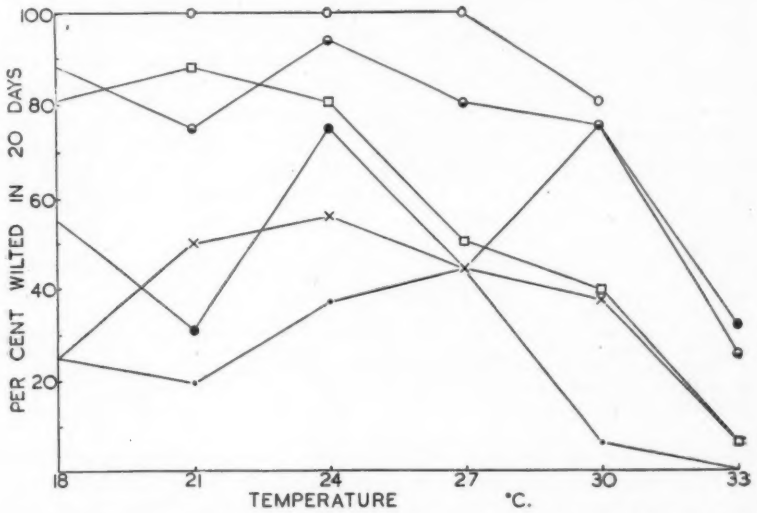


FIG. 10. *Disease incidence under varying conditions of soil temperature in soil infested with six strains of the muskmelon Fusarium.* ○ = *Wild type.* ◒ = *Ultra-violet mutant.* □ = *Dark semiraised.* × = *Intermediate appressed.* ● = *Light appressed.* • = *Very light appressed.*

In the next and final experiment an additional set of inoculated cans was included. The soil from one of the two check cans in each tank was sterilized and inoculum of the wild type was incorporated into this soil in the usual manner. It is apparent from Fig. 11 that disease incidence was more severe in the soil freshly inoculated with the wild type than in that which had been inoculated five weeks previously. It is likely, in view of experiments already described, which demonstrated a depression of disease incidence in non-sterile soil, that the factor involved here was the competition of other organisms that had become established in the cans during the preceding experiment. The same factor was probably responsible for the fact that, in general, the mutant strains seemed less virulent in the second planting.

The relative degree of disease incidence induced by the mutant strains throughout these two experiments does not seem consistent. This is not surprising in view of the fact that the competition factor may not have been

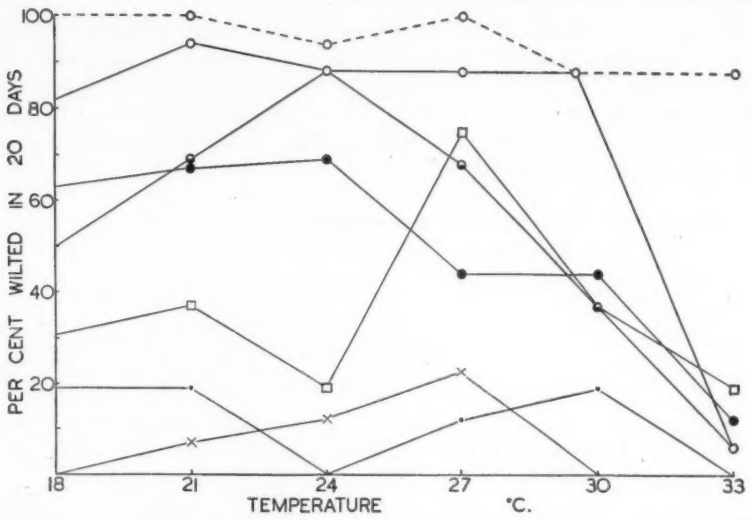


FIG. 11. *Disease incidence under varying conditions of soil temperature in soil infested with six strains of the muskmelon wilt Fusarium.* ○——○ = *Wild type.* ○ - - - - ○ = *Wild type, freshly inoculated into sterilized soil.* ◒ = *Ultra-violet mutant.* □ = *Dark semiraised.* × = *Intermediate appressed.* ● = *Light appressed.* • = *Very light appressed.*

uniform. Moreover the possibility exists that certain strains are more sensitive to the operation of this factor than others. It is also possible that the effect of temperature was operating through the competition factor. The observations of Henry (3) regarding the effect of temperature and soil sterilization on the reaction of wheat seedlings to *Ophiobolus graminis* Sacc. are of interest in this connection. He found that at 13° C. disease incidence was equally severe in both sterilized and unsterilized soils, but at 27° C. it was less severe in unsterilized.

The importance of paying close attention to all possible changes of environment during such experiments is evident from the variance between the results obtained in the last two experiments. In general, however, these agree with the preceding two in indicating a decline in disease incidence toward the higher soil temperatures, but there was also a suggestion of a decline at the lower end of the temperature range.

Since it is highly improbable that any of the cultural variants employed in the experiments are of significance in the field pathology of the disease, their inclusion in a temperature experiment is mainly of academic interest. When making studies of disease incidence under controlled conditions for com-

parison with field observations, it is imperative that great care should be taken to ensure that any laboratory inoculum employed consists exclusively of the organism as it occurs in the field, i.e. the wild type.

(*e*) *The Effect of High and Low Soil Moistures on Disease Incidence*

During the summer of 1943 an experiment was performed to determine the effect of soil moisture on disease incidence under different conditions from those of the temperature tanks.

Four flats of sterilized soil were mixed thoroughly and then halved. One-half was inoculated in the proportion of 1% by volume with cornmeal–sand medium on which the wild type strain had grown for 20 days. The soil was placed in flats that were planted with 140 seeds each and weighed. To two of the flats, one of which had not been inoculated, an additional litre of water was added and this sufficed to make the soil very moist. Soil moisture determinations indicated moisture contents of 38 and 62% of the moisture holding capacity at the low and high moistures, respectively. These moisture levels were maintained by periodic weighings with the addition of water to make up the original weight. During the experiment the soil temperature in the flats varied from 14° to 31° C.

TABLE III

DISEASE INCIDENCE AND HOST GROWTH AT HIGH AND LOW SOIL MOISTURE LEVELS

Soil moisture	Wilt in 15 days, %	Wilt in 20 days, %	Average weight of seedlings in check flats in 20 days, gm.
Low	65	90	0.62
High	33	78	1.28

The results of this experiment are summarized in Table III. Disease incidence was greater at low soil moisture. The seedlings, however, showed much better growth at the high soil moisture. A correlation is thus evident between unfavourable conditions for host growth and high disease incidence, suggesting that the factor involved may be a predisposition of the host. The possibility exists nevertheless, that high soil moisture may suppress disease incidence by accentuating the competitive factor. These results compare well with the temperature experiment previously described in which seeds were planted directly in the cans.

Unfortunately, no field experiments are available to correlate with the results described here. However, the effect of moisture noted seems to be general among pathogenic species of *Fusarium*. Tisdale (10) has shown this to be the case in the cabbage wilt *Fusarium*. It is not general among soil-inhabiting fungus parasites since McKeen (5) has found greater disease incidence at high soil moistures with *Verticillium*.

(f) The Effect of Seed Treatment on Disease Incidence in Heavily Infested Soil

The seed treatment adopted to avoid a seed rot caused in unsterilized soil by other organisms than the wilt *Fusarium* has been described previously in connection with the dilution experiments. This treatment has been found highly effective in preventing pre-emergence blight caused by the wilt *Fusarium* in heavily infested soil, a symptom that is commonly noted when inoculum is added to freshly sterilized soil.

Enough sterilized soil for four flats was mixed thoroughly. Half of this was then inoculated with a 1% concentration of cornmeal–sand medium on which the wild type strain was growing. The soil was placed in flats, two of which served as checks. Each flat was planted immediately with 140 seeds of which 70 had been treated as follows. The seeds were first soaked for three hours in tap water and were then spread out on papers until the surfaces were dry. They were then shaken with sufficient Semesan to provide a heavy protective coat of the dust. The results of the experiment are summarized in Table IV.

TABLE IV

THE EFFECT OF SEED TREATMENT ON DISEASE INCIDENCE IN HEAVILY INFESTED SOIL

Seed	Emergence, %	Mortality in 15 days, %	Mortality in 20 days, %
Treated	86, 90	41, 43	83, 85
Untreated	60, 60	100, 100	100, 100

This table shows that seed treatment had a pronounced protective effect and this is further demonstrated by Fig. 6. There was no evidence of pre-emergence blight among the treated seeds. Even in the checks the treated seed showed a slightly higher emergence than the untreated: 87% as compared with 83%.

The suppression of pre-emergence blight by this treatment can be explained on the assumption that the organic mercury fungicide imposes a protective barrier between the germinating seed and the parasite. It is difficult to account for the continuance of this protective effect as late as 20 days after planting. Possibly the germinating seed represents a very susceptible stage in the life of the host and it is better able to endure the later attacks of the parasite. There is the additional possibility that invasion is frequently accomplished at the base of the hypocotyl, which remains in the protected area in the vicinity of the treated seed coat. Microscopic studies on diseased seedlings have suggested that invasion often does take place at this point.

A similar effect has been reported with the watermelon *Fusarium* by Porter and Melhus (8). By treating seed with Bayer dust and Semesan they were able to increase the emergence values in heavily infested soil from 7% to 81 and 99%, respectively.

(*g*) *Experiments Dealing With Host Range*

(*i*) *Infection Experiment with Five Cucurbit Species*

The reaction of five cucurbit species to four strains of the parasite was tested. The plants employed were: muskmelon (Bender's Surprise); watermelon (Winter Queen); cucumber (Straight 8); marrow (Green Trailing); squash (Delicious).

Flasks of cornmeal–sand medium were inoculated separately with the following strains of the organism: the wild type and the dark semiraised, the intermediate appressed, and the light appressed mutants. Nine days later the inoculum of each strain was incorporated in the proportion of 1% by volume into sufficient sterilized 'sandy knoll' soil to make two flats. Two non-inoculated flats served as checks. Each pair of flats contained 42 seeds of muskmelon, watermelon, and cucumber and 15 seeds of marrow and squash.

The muskmelons showed symptoms of wilt as early as eight days after planting, but 25 days later no wilt was evident among the other cucurbits. By this time less than 10% of the muskmelons remained alive. It is thus evident that the host range of the pathogen is narrow. Since the three mutants were also pathogenic to muskmelons only, the results of this experiment do not support the thesis that the mutants represent phases in the sphere of variability of an organism the variants of which are pathogenic on different hosts.

(*ii*) *Three Botanical Varieties of Muskmelon and Five Strains of Fusarium*

The botanical varieties employed were Bender's Surprise (included in *Cucumis Melo* var. *reticulatus* Naud.), the Vine-Peach or Mango Melon (*Cucumis Melo* var. *chito* Naud.), and the Banana muskmelon, which is considered by the authors of *The Cucurbits* (9) to be intermediate in many respects between the common muskmelon (*Cucumis Melo* var. *reticulatus* Naud.) and *Cucumis Melo* var. *flexuosus* Naud.

The flats of 'sandy knoll' soil employed in this experiment had been sterilized and inoculated a month previously with inoculum of the wild type and the dark semiraised, the intermediate appressed, light appressed, and very light appressed mutants in connection with an experiment performed to compare the virulence of these strains. A non-inoculated flat of sterilized 'sandy knoll' soil served as check. Forty-two seeds of each variety were planted per flat.

Table V summarizes the data of this experiment. It is apparent that Bender's Surprise was susceptible whereas the Banana muskmelon was definitely less susceptible. The Vine-Peach was resistant and this was true with all five strains of the organism. On all three varieties the highest disease incidence was observed in the flat inoculated with the wild type, which indicates that the wild type is the strain best suited for resistance studies.

(*iii*) *Twelve Melon Varieties and two Strains of Fusarium*

This experiment included, in addition to the three botanical varieties of (*ii*), nine horticultural varieties of muskmelon (*Cucumis Melo* var. *reticulatus*

TABLE V

COMPARISON OF THE RESISTANCE OF THREE MUSKMELON VARIETIES TO FIVE STRAINS OF THE PARASITE

Strain	Wilt in 20 days, %		
	Bender's Surprise	Banana muskmelon	Vine-Peach
Wild type	100	75	25
Dark semiraised	69	47	12
Intermediate appressed	32	23	0
Light appressed	38	31	3
Very light appressed	3	6	0

Naud.). Two of these, 10-38 and 11-38, were developed at the Minnesota Agricultural Experiment Station; 38-3-05, 40-8-*F* and 38-4-06-*F* are three partially resistant lines developed by Mr. O. J. Robb at the Vineland Experiment Station. The remaining four are common commercial varieties: Hale's Best, John the Gardiner, Honey Rock, Honey Gold.

The artificially infested soil in this experiment had already been employed in the dilution experiment described under (*b*) above. Sterilized and unsterilized soil containing inoculum of the wild type strain were mixed thoroughly. Twelve flats were filled with this soil and 10 with soil treated similarly but containing inoculum of the light appressed strain.

The disease incidence as shown in Table VI varied considerably among the 12 varieties. The Vine-Peach appeared to be the most resistant here as in the

TABLE VI

THE REACTION OF 12 HORTICULTURAL VARIETIES OF MELON TO TWO STRAINS OF *Fusarium*

Variety	Percentage wilt			
	17 days		37 days	
	Strain			
	Wild type	Light appressed mutant	Wild type	Light appressed mutant
Bender's Surprise	57	39	100	99
10-38	45	19	70	89
11-38	22	17	58	34
38-3-05	76	44	90	46
Honey Gold	59	31	76	56
Vine-Peach	1	0	51	35
Banana Muskmelon	37	23	95	91
Hale's Best	11	11	97	95
John the Gardiner	30	30	97	87
Honey Rock	39	15	97	96
40-8-*F*	44.5	—	97	—
38-4-06-*F*	44.5	—	88	—

previous experiment. Disease incidence after 37 days was greatest in Bender's Surprise but this was not the case at 17 days, when 38-3-05 and Honey Gold seemed equally or more susceptible. In general, all the varieties were more susceptible to the wild type than to the light appressed mutant.

(*iv*) *Infection Experiment With Some European and Asiatic Varieties of Muskmelon*

Two flats of sterilized soil that had been inoculated in the usual manner with the wild type and employed in a previous experiment were mixed thoroughly and planted with seeds of several muskmelon varieties. These included three Indian varieties: Irani Sarda, Quetta Late, and Duki Garma Early. The systematic position of these varieties is uncertain. They have not been grown to maturity but the seeds of all three are large and the cotyledons of the young seedlings are also much larger than in any other variety tested. In *The Cucurbits* (9) a race of large-fruited, strong growing Persian melons is mentioned as allied to *Cucumis Melo* var. *reticulatus* Naud. It is possible that the Indian varieties may be related to these.

The variety Edible Gold was obtained from a commercial seed company (McFayden) and is stated to be from Poland. An English muskmelon, variety unknown, was included in the experiment and a number of seeds of Bender's Surprise were planted in each flat for comparison. A total of 100 seeds was planted per flat.

From Table VII it is evident that disease incidence was greatest in Bender's Surprise, Edible Gold, and the English melon. It was relatively low in the three Indian varieties. Although the latter also showed rather low emergence values, it is unlikely that this was due to pre-emergence blight since similar emergence values were observed in the check flat.

TABLE VII

COMPARISON OF DISEASE INCIDENCE IN SOME VARIETIES OF EUROPEAN AND ASIATIC MELONS

Variety	Number of seeds planted	Emergence, %	Wilt in 16 days, %
Irani Sarda	40	65	38
Quetta Late	20	60	17
Duki Garma Early	10	50	20
Edible Gold	20	75	73
English Melon	40	95	82
Bender's Surprise	70	78	95

(*v*) *Comparison of the Resistance of Three Varieties in Staked Rows in Greenhouse Soil*

As a check on the resistance experiments performed in flats of soil, the resistance of three varieties was tested in greenhouse plantings. Seeds were planted in sterilized soil in 4-in. pots and two weeks later the resulting plants

were thinned to two per pot and transplanted as carefully as possible to holes of proper depth in greenhouse soil. Each row contained nine pots of melons, all of the same variety. The varieties employed here were Bender's Surprise, 10-38, and 38-3-05. A year previously oat-hull medium on which the wild type was growing had been scattered over the surface of this soil following steam sterilization by the tile method.

Symptoms of wilt were noted in some of the Bender's Surprise plants 15 days after transplanting. Two months later all but one of the 18 Bender's Surprise plants had wilted. No wilt symptoms were visible in either of the other varieties.

The expression of resistance was much sharper here than in the previous experiment in which the resistance of these varieties was compared in flats. For example, as shown in Table VI, after 37 days in the case of the wild type strain, 10-38 showed 30% less wilt than Bender's while 38-3-05 showed only 10% less. These results would indicate that when searching for resistant varieties, even small indications of resistance in flat experiments make further investigation advisable under more natural conditions of environment.

The observations of this experiment are of some significance with regard to the problem of breeding for resistance. It seems unnecessary to begin with a highly resistant but almost inedible variety like the Vine-Peach when melons are available that possess both horticultural quality and resistance to *Fusarium*.

Discussion

The effect of temperature on disease incidence noted during these studies is in general agreement with that reported by Leach and Currence (4) for the Fusarium wilt of muskmelons in Minnesota. They noted a definite decrease in severity at temperatures above 30° C., which has also been found here. Further, in both instances no minimum temperature was found below which the host grew and escaped infection. Leach and Currence point out that this is in contrast to most other Fusarium wilts whose reactions to soil temperature have been studied. Since low soil moisture tends to accentuate disease incidence, the indication is that wilt may be particularly serious at temperatures below 30° C. during dry weather. At the Vineland Experiment Station during July, 1943, the soil temperature at a depth of four inches varied between the extremes of 17° and 26° C. which is well within the range found to favour the attack of the parasite. It is doubtful whether soil temperatures in Ontario ever remain sufficiently high to cause the decrease in disease incidence noted at temperatures above 30° C. High summer temperatures, moreover, are usually associated with dry weather and as a consequence the effect of soil moisture would tend to favour the pathogen, although the experiments suggested that this effect is not as marked with mature plants as with seedlings.

Disease incidence was found to be consistently less severe in unsterilized soil and the opinion is expressed that the factor responsible is biological. This appears to be the general experience with soil-inhabiting pathogens.

Since the literature in this field has been reviewed by Waksman (12) and by Garrard and Lochhead (2), no effort will be made to cover it here.

It appears that the most important factor in controlling the disease is the protective effect noted in unsterilized naturally infested soil. Unfortunately, this effect has been found associated with a stunting, at least in the case of seedlings. It is possible that stunting is not as severe in older plants but no data in this regard are available. A further investigation of this phase of the problem would be advisable and should include a thorough study of the soils of the melon-growing areas with regard to the distribution of the wilt organism, the protective effect, and the stunting.

The experiments described under Part I of this series (6) indicated that variants of the types that commonly appeared in culture occur rarely, if ever, in nature. Further, when inoculated into a field plot artificially they caused less disease incidence and were less able to survive as a disease factor than the parent wild type. In the present studies the wild type was found to be more virulent on all host varieties and under varying soil temperatures than any of the cultural variants that were tested. It thus seems reasonable to regard these mutant strains as merely cultural phenomena and to advise employing only the wild type strain when infesting soil for resistance trials. The alternative view, that the cultural variants may represent forms displaying selective pathogenicity toward various varieties of the host, is not supported by the data obtained during these studies. Likewise, Wellman and Blaisdell (15) failed to find evidence of selective pathogenicity among variants of the tomato wilt *Fusarium*.

It is possible that the concept of the 'wild type' may be instrumental in introducing a further degree of clarity into the problem of breeding for resistance to Fusarium diseases, a field that appears somewhat confused by cultural variation in the causal organisms. For example, although Wellman and Blaisdell (15, 16) showed that what they term the *R*-type was consistently high in virulence and all the other types could be obtained from it, they do not consider the possibility that only this type may have existed in the field. A number of isolates of the tomato wilt *Fusarium* that had been maintained in culture over a period of years were purified by monosporous isolations and compared. Many of these showed wide differences in their cultural characteristics and it was assumed that they had occurred as definite strains in the field. The experience of the writer with the muskmelon *Fusarium* has indicated the extreme difficulty of maintaining the wild type in culture over a period of time without careful repurification to prevent its being crowded out by mutants that are more aggressive in culture than the parent. While it is perhaps unwise to generalize too freely from the behaviour of a single member of the genus, it seems not unreasonable that one way to fail to recognize initial similarity among isolates of a *Fusarium* is to grow them in culture for a number of years before making the comparison. This is what Wellman and Blaisdell have done. The variant types they describe, with the implication that they exist as such in the field, are, in general, quite similar

to the range of mutant types obtained from the muskmelon *Fusarium* in Part I of the present studies. In fact the resemblance was so striking that part of the nomenclature they employed ("raised," "appressed") was found very apt and was adopted for the variants of the muskmelon *Fusarium*. They concluded further (16) that since the *R*-type tends to lose its virulence in culture owing to the appearance of milder strains, this process must occur in nature as well. This leads them to the additional conclusion that there is also a build-up of virulence in progress to balance the loss: "Such variation must take place in nature, since a wholly downward trend would eventuate in complete deterioration of a highly pathogenic population, which evidently does not happen in the field." The observations in the present series on the muskmelon *Fusarium* present a simpler explanation, that whereas the wild type is readily displaced in culture by less virulent mutants, in the field it is genetically stable. In fact, when such mutants were actually introduced into the field along with the wild type, after a year they were found to be negligible as a disease factor but the wild type still caused wilt. It is evident that the continuous build-up of virulent strains visualized by Wellman and Blaisdell (16) and by Wellman (14) would present an almost hopeless problem from the viewpoint of the plant breeder; this does not seem to be the case in practice since most Fusarium-resistant varieties maintain their resistance well over a period of years.

As a fundamental part of any breeding program dealing with a soil *Fusarium* it would seem advisable to determine whether the organism occurs in the field throughout the area involved as a single, uniform wild type. Although this may readily be investigated by making a large number of isolates from dispersed points in the area and comparing them immediately after isolation, studies of this nature are rare in the literature on *Fusarium*. Weindling (13) found that all the original isolates from plants diseased with *Fusarium vasinfectum* Atk. were alike and had abundant aerial mycelium, and Ullstrup (11) collected perithecia of *Gibberella Saubinetii* (Mont.) Sacc. from Illinois, Iowa, and Minnesota and found all the original isolates to be identical in culture. There can be little doubt, however, that more than one wild type may occur in a given region since Nelson *et al.* (7) have described two forms of the celery yellows pathogen in Michigan. Symptoms characteristic of each were recognizable in the field. Both forms produced abundant aerial mycelium and could be distinguished with difficulty in culture. If it is found that all the original isolates of a given Fusarial pathogen are similar in culture, the possibility that a number of pathogenic strains are included might then be investigated. For example, Broadfoot (1) found nine forms of *Fusarium Lini* Bolley among isolates from various localities as distinguished by their relative virulence on four flax varieties.

In brief, the results obtained during the present series of studies on the *Fusarium* of muskmelon wilt may have an immediate bearing on certain problems of a practical nature. The advisability of including a critical cultural study of the pathogen in any program of breeding for resistance to a

Fusarium disease is emphasized, and this becomes of especial importance when, for any reason, laboratory inoculum must be employed.

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